Patent
Attorney's Docket No. 032396-043

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY PATENT
APPLICATION TRANSMITTAL LETTER

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Assistant Commissioner for Patents
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Sir:

Enclosed for filing is the utility patent application of Jim A. Wright, Aiping H. Young and Yoon S. Lee for NEUROPILIN ANTISENSE OLIGONUCLEOTIDE SEQUENCES AND METHODS OF USING SAME TO MODULATE CELL GROWTH .

Also enclosed are:

[X] 12 sheet(s) of [ ] formal [X] informal drawing(s);

[ ] a claim for foreign priority under 35 U.S.C. §§ 119 and/or 365 is [ ] hereby made to _____ filed in _____ on _____; [ ] in the declaration;

[ ] a certified copy of the priority document;

[ ] a Constructive Petition for Extensions of Time;

[ ] _____ statement(s) claiming small entity status;

[ ] an Assignment document;

[ ] an Information Disclosure Statement; and

[ ] Other: _____.

The declaration of the inventors (un-executed) is also enclosed. The executed declaration of the inventors will follow.

The filing fee has been calculated as follows:

CLAIMS					
	NO. OF CLAIMS		**EXTRA CLAIMS**	**RATE**	**FEE**
Basic Application Fee					$ 760.00
Total Claims	16	MINUS 20 =	0	x $18.00	$ .00
Independent Claims	6	MINUS 3 =	3	x $78.00	$ 234.00
If multiple dependent claims are presented, add $260.00					.00
Total Application Fee					$994.00
If verified Statement claiming small entity status is enclosed, subtract 50% of Total Application Fee					
Add Assignment Recording Fee of $40.00 if Assignment document is enclosed					
TOTAL APPLICATION FEE DUE				**(NOT ENCLOSED)**	**$994.00**

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PATENT
Attorney Docket No. 032396-043

BE IT KNOWN, that WE, JIM A. WRIGHT, a resident of Toronto, Canada, AIPING H. YOUNG, a resident of Toronto, Canada and YOON S. LEE, a resident of Don Mills, Canada, have invented new and useful improvements in:

NEUROPILIN ANTISENSE OLIGONUCLEOTIDE SEQUENCES AND METHODS OF USING SAME TO MODULATE CELL GROWTH

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/082,791 filed on April 23, 1998, which is incorporated by reference herein in its entirety.

PATENT
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NEUROPILIN ANTISENSE OLIGONUCLEOTIDE SEQUENCES AND METHODS OF USING SAME TO MODULATE CELL GROWTH

REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 60/082,791 filed April 23, 1998, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to oligonucleotides that are complementary to mammalian neuropilin (or $VEGF_{165}R$) mRNA which oligonucleotides modulate cell growth in mammals. This invention is also related to methods of using such compounds in inhibiting the growth of tumor cells in mammals and to inhibit angiogenesis in mammals. This invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

References

The following publications, patent applications and patents are cited in this application as superscript numbers:

1. Tischer, E., et al., "The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing," *J Biol Chem.* **266***:* 11947-54, (1991).

2. Poltorak, Z., et al., " $VEGF_{145}$, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix", *J Biol Chem.* **272***:* 7151-8, 1997.

3. Terman, B. I., et al., "Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor", *Biochem Biophys Res Commun.* **187***:* 1579-86, 1992.

4. Millauer, B.,et al., "High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis", *Cell.* **72***:* 835-46, 1993.

5. Shibuya, M., et al., "Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family", *Oncogene.* **5**: 519-24, 1990.

6. de Vries, C., et al., "The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor", *Science.* **255***:* 989-91, 1992.

7. Kawakami, A., et al., "Developmentally regulated expression of a cell surface protein, neuropilin, in the mouse nervous system", *J Neurobiol.* **29***:* 1-17, 1996.

8. Takagi, S., et al., "Expression of a cell adhesion molecule, neuropilin, in the developing chick nervous system", *Dev Biol.* **170***:* 207-22, 1995.

9. Soker, S., et al., "Neuropilin-1 is expressed by endothelial and tumor cells as an isoform- specific receptor for vascular endothelial growth factor", *Cell.* **92***:* 735-45, 1998.

10. Soker, S., et al., "Inhibition of vascular endothelial growth factor (VEGF)-induced endothelial cell proliferation by a peptide corresponding to the exon 7- encoded domain of $VEGF_{165}$", *J Biol Chem.* **272***:* 31582-8, 1997.

11. He, Z. and Tessier-Lavigne, M. "Neuropilin is a receptor for the axonal chemorepellent Semaphorin III", *Cell.* **90***:* 739-51, 1997.

12. Mitsuhashi, M. "Strategy for designing specific antisense oligonucleotide sequences", *J Gastroenterol.* **32**: 282-7, 1997.

13. Alama, A., et al., "Antisense oligonucleotides as therapeutic agents", *Pharmacol Res.* **36**: 171-8, 1997.

14. Curcio, L. D., et al., "Oligonucleotides as modulators of cancer gene expression", *Pharmacol Ther.* **74**: 317-32, 1997.

15. Brem, S., et al., "Prolonged tumor dormancy by prevention of neovascularization in the vitreous", *Cancer Res.* **36**: 2807-12, 1976.

16. Holmgren, L., et al., "Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression", *Nat Med.* **1**: 149-53, 1995.

17. Parangi, S., et al., "Antiangiogenic therapy of transgenic mice impairs de novo tumor growth, *Proc Natl Acad Sci U S A.* **93**: 2002-7, 1996.

18. Choy et al., "Molecular mechanisms of drug resistance involving ribonucleotide reductase: hydroxyurea resistance in a series of clonally related mouse cell lines selected in the presence of increasing drug concentrations" *Cancer Res.* 48:2029-2035 (1988)

19. Fan et al., "Ribonucleotide reductase R2 component is a novel malignancy determinant that cooperates with activated oncogenes to determine transformation and malignant potential" *Proc. Natl. Acad. Sci* USA 93:14036-40 (1996)

20. Huang and Wright, "Fibroblast growth factor mediated alterations in drug resistance and evidence of gene amplification" *Oncogene* 9:491-499 (1994)

21. International Patent Application Publication No. WO99/02556, "Semaphorin Receptors"

22. International Patent Application Publication No. WO99/04263, "Semaphorin Receptor"

23. *Remington's Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia PA 17th ed. (1985)

24. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1989, 1992)

25. Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore Maryland (1989)

26. Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988)

27. Hurta and Wright, "Malignant transformation by H-ras results in aberrant regulation of ribonucleotide reductase gene expression by transforming growth factor-beta" *J. Cell Biochem* **57**:543-556 (1995)

28. International Patent Application Publication No. WO97/21808, "Modified VEGF Antisense Oligonucleotides"

29. Nielsen et al.; *Science* (1991) **354**:1497

30. Good and Nielsen; "Inhibition of translation and bacterial growth by peptide nucleic acid targeted to ribosomal RNA", *PNAS USA* (1998) **95**:2073-2076

31. Buchardt, deceased, et al., U.S. Patent No. 5,766,855

32. Buchardt, deceased, et al., U.S. Patent No. 5,719,262

33. U.S. Patent No. 5,034,506

34. Altschul, et al. "Basic local alignment search tool", *J. Mol. Biol.* (1990) **215**:403-10;

35. Devereux J. et al., "A comprehensive set of sequence analysis programs for the VAX", *Nucleic Acids Res.* (1984) **12**:387-395;

36. Chang et al.; Somatic Gene Therapy, CRC Press, Ann Arbor MI (1995);

37. Vega et al.; *Gene Targeting*, CRC Press, Ann Arbor MI (1995)

38. *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988)

39. Sullivan, U.S. Patent No. 5,225,347

40. U.S. Patent 5,023,252, issued June 11, 1991

41. Felgner et al.,U.S. Patent No. 5,580,859

42. Dreeley et al., Science, 258:1650-1654 (1992)

43. Uhlmann et al. *Chem Rev.* 90:534-583 (1990)

44. Agrawal et al. *Trends Biotechnol.* 10:152-158 (1992)

45. Smith et al., (1994) *Invest. Ophthalmol. Vis. Sci* 35:101-111

46. Pierce et al., (1995) *Proc. Natl. Acad. Sci* USA **92**:905-9

All of the above publications, patent applications and patents are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

Proliferation of new capillaries, called angiogenesis or neovascularization, is critical for the transition of a small localized tumor to expand into a large malignant growth. Without the appropriate development of blood supply, tumor growth is dramatically impaired.

Neovascular diseases of the retina such as diabetic retinopathy, retinopathy of prematurity and age-related macular degeneration are a major cause of blindness in the United States and the world. During the course of diabetes mellitus, the retinal vessels undergo changes that result in not only leaky vessels but also vessel drop out resulting in retinal hypoxia. One of the effects of this is neovascularization of the retina resulting in bleeding and retinal detachment. Retinopathy of prematurity is a common cause of blindness in children. The blood vessels of the retina cease to develop into the peripheral retina resulting in ischemia and localized hypoxic conditions as the metabolic demands of the developing retina increase. The resulting hypoxia stimulates the subsequent

neovascularization of the retina which can lead to irreversible vision loss. Ocular neovascularization is also the underlying pathology in sickle cell retinopathy, neovascular glaucoma, retinal vein occlusion and other hypoxic diseases. Recent experimental data show a high correlation between vascular endothelial growth factor expression and retinal neovascularization. (28)

Of numerous angiogenic factors produced from tumor cells, vascular endothelial growth factor (VEGF) is shown to be a major mediator of tumor angiogenesis and neovascularization. Human VEGF monomers exist as five different isoforms, among which $VEGF_{121}$ and $VEGF_{165}$ are most abundant (1, 2). VEGF activities are exerted by its binding to high affinity tyrosine kinase receptors present on endothelial cells lining tumor vasculature. Two such receptors have been isolated: KDR/Flk-1(3, 4) which appears to be the major transducer of VEGF signals and Flt-1(5, 6).

Neuropilin or $VEGF_{165}R$ or the vascular endothelial growth factor receptor, which was originally isolated as a receptor for the collapsin/semaphorin that mediates neuronal cell guidance (7, 8), has been recently cloned as a new isoform specific receptor expressed by endothelial cells for $VEGF_{165}$ (9). The nucleic acid sequence for human neuropilin has been reported (9, 11, 21, 22). Neuropilin acts as a coreceptor for $VEGF_{165}$ binding to KDR/Flk-1 and modulating subsequent bioactivity, i.e. tumor-induced angiogenesis. It is also highly expressed in tumor derived cells such as MDA-MB-231 breast carcinoma cells and PC3 prostate carcinoma cells, among the few tested (9, 10). VEGF has also been shown to bind to Hela, melanoma and NIH 3T3 cells.

Antisense technology has been widely adopted not only as a useful research tool (12), but also as a rational approach to acquire new therapeutic compounds for the treatment of many human diseases including cancer (13, 14). Antisense

oligonucleotides can specifically hybridize to mRNA sequences and inhibit expression of proteins that are important in initiation and/or progression of human cancer. Therefore, it would be desirable to identify antisense oligonucleotides directed against neuropilin which act to inhibit the expression and production of neuropilin/$VEGF_{165}R$ with higher specificity and with less toxicity.

SUMMARY OF THE INVENTION

This invention is directed to antisense oligonucleotides which modulate the expression of the neuropilin genes and production of neuropilin/$VEGF_{165}R$ in mammals and pharmaceutical compositions comprising such antisense oligonucleotides. This invention is also related to methods of using such antisense oligonucleotides for inhibiting the proliferation of new capillaries or angiogenesis or neovascularization involved in tumor growth and metastasis in mammals.

Accordingly, in one of its composition aspects, this invention is directed to an antisense oligonucleotide from about 3 to about 100 nucleotides, comprising nucleotides complementary to the neuropilin mRNA of a mammal. The antisense oligonucleotide may be nuclease resistant and may have one or more phosphorothioate internucleotide linkages. The antisense oligonucleotide may further comprise additional nucleotides which are not complementary to the neuropilin mRNA.

In another of its composition aspects, this invention is directed to an antisense oligonucleotide from about 20 to about 100 nucleotides, comprising a sequence selected from the group consisting of SEQ ID NOs: 1 - 30 set forth in Table 1 which oligonucleotide inhibits neuropilin expression.

In another of its composition aspects, this invention is directed to a vector comprising an oligonucleotide sequence from about 20 to 100 nucleotides comprising a sequence selected from the group consisting of SEQ ID NOs:1 - 30 as set forth in Table 1 which oligonucleotide inhibits neuropilin expression.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an antisense oligonucleotide from about 20 to about 100 nucleotides comprising a sequence selected from the group consisting of SEQ ID NOs: 1 - 30 as set forth in Table 1 which oligonucleotide inhibits neuropilin expression.

In one of its method aspects, this invention is directed to a method for inhibiting the growth of a mammalian tumor comprising, administering to a mammal suspected of having the tumor an effective amount of an antisense oligonucleotide from about 3 nucleotides to about 100 nucleotides comprising a sequence complementary to mammalian neuropilin mRNA under conditions such that the growth of the tumor is inhibited. The antisense oligonucleotide may be administered with a chemotherapeutic agent.

In another of its method aspects, this invention is directed to a method for inhibiting the metastasis of a mammalian tumor comprising, administering to a mammal suspected of having a metastatic tumor an effective amount of an antisense oligonucleotide from about 3 nucleotides to about 100 nucleotides comprising a sequence complementary to mammalian neuropilin mRNA under conditions such that the metastasis of the tumor is inhibited. The antisense oligonucleotide may be administered with a chemotherapeutic agent.

In another of its method aspects, this invention is directed to a method for inhibiting angiogenesis or neovascularization in a mammal comprising, administering to a mammal an effective amount of an antisense oligonucleotide from about 3 nucleotides to about 100 nucleotides complementary to mammalian neuropilin mRNA under conditions such that neovascularization is inhibitied.

In another of its method aspects, this invention is directed to a method for inhibiting neuropilin expression comprising contacting nucleic acid specific for neuropilin with an antisense oligonucleotide from about 20 nucleotides to about 100 nucleotides comprising a sequence selected from the group consisting of SEQ ID NOs: 1 - 30 as set forth in Table 1 which oligonucleotide inhibits neuropilin expression.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A - F are graphs of the percentage of inhibition of the colony forming ability of different cell lines by administration of the indicated antisense oligonucleotides. Fig 1A shows the percentage inhibition of the human melanoma cell line C8161; Fig. 1B shows percentage inhibition of the human lung cancer cell line A549; Fig. 1C shows the percentage inhibition of the human melanoma cell line A2058: Fig. 1D shows the percentage inhibition of the human colon cancer cell line HT-29; Fig. 1E shows the percentage inhibition of the human prostate cancer cell line PC-3; and Fig 1F shows the percentage inhibition of the human pancreatic cancer cell line AsPC-1.

Figs. 2A and 2B are autoradiographs of Northern Blots of RNA from either human melanoma cancer cell line A2058 (Fig. 2B) or human breast cancer cell line MDA-MB-231 (Fig. 2A) after administration with one of the following antisense oligonucleotides: GTI3601 [SEQ ID NO:1]; GTI3602 [SEQ ID NO:2]; GTI3603

[SEQ ID NO:3]; GTI3604 [SEQ ID NO:4]; GTI3610 [SEQ ID NO:10]; GTI3611 [SEQ ID NO:11]; and GTI3612 [SEQ ID NO:12].

Fig. 3A is a graph of the volume of a tumor over time following injection of human HT-29 colon cancer cells into the right flank of mice with administration of antisense oligonucleotide GTI3602 [SEQ ID NO:2] or without (saline).

Fig. 3B is a graph of the weight of a tumor 20 days after injection of human HT-29 colon cancer cells into the right flank of mice with administration of antisense oligonucleotide GTI3602 [SEQ ID NO:2] or without (saline).

Fig. 4 is a graph of the average number of lung metastases per mouse by the human melanoma cell line C8161 after treatment of the cell line with the antisense oligonucleotides GTI3611 [SEQ ID NO:11] or GTI3602 [SEQ ID NO:2] or without [control].

Fig. 5 is the nucleotide sequence of human neuropilin cDNA. [SEQ ID NO:33].

Fig. 6 is the nucleotide sequence of rat neuropilin cDNA. [SEQ ID NO:34].

Fig. 7 is the nucleotide sequence of mouse neuropilin cDNA. [SEQ ID NO:35].

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to oligonucleotides complementary to mammalian neuropilin mRNA which oligonucleotide modulate cell growth.

Neuropilin is a receptor for vascular endothelial growth factor or VEGF. VEGF has been found to modulate tumor induced angiogenesis. Neuropilin is also highly expressed in tumor derived cells such a MDA-MB-231 breast carcinoma cells and in tissue culture cells such as Hela and NIH 3T3 cells. This suggests that, in addition to its role in angiogenic stimulation, neuropilin may act, in an autocrine manner, as a sole signal transducer for VEGF activities on tumor cells themselves by enhancing survival, differentiation, or motility. Another possibility may be that neuropilin has storage or sequestration function.

Definitions:

As used herein, the following terms have the following meanings:

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complementary to the desired mRNA. Preferably, the antisense oligonucleotide is complementary to that portion of a mammalian neuropilin mRNA or $VEGF_{165}R$ mRNA that effectively acts as a target for inhibiting neuropilin expression. It is contemplated that the antisense oligonucleotide may be complementary to any of the 5' untranslated region of the mRNA, the coding region or the 3' untranslated region of the mRNA. Most preferably, the antisense oligonucleotide is complementary to the nucleotide sequence set forth in Fig. 5.

Without being limited to any theory or mechanism, it is generally believed that the activity of antisense oligonucleotides depends on the binding of the oligonucleotide to the target nucleic acid (e.g. to at least a portion of a genomic

region, gene or mRNA transcript thereof), thus disrupting the function of the target, either by hybridization arrest or by destruction of target RNA by RNase H (the ability to activate RNase H when hybridized to RNA) resulting in inhibition of neuropilin expression.

The term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and inter-sugar (backbone) linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligomers may be preferred over naturally occurring forms because of the properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease resistance, increased uptake into cells) or two or more oligonucleotides of the invention may be joined to form a chimeric oligonucleotide.

The antisense oligonucleotides of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring or synthetic monomeric bases, including adenine, guanine, cytosine, thymine and uracil. The oligonucleotides may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine. The modifications may

also include attachment of other chemical groups such as methyl, ethyl, propyl groups to the various parts of the oligonucleotides including the sugar, base or backbone components.

The antisense oligonucleotides of the invention may also comprise modified phosphorus oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatom or heterocyclic intersugar linkages. For example, the antisense oligonucleotides may contain methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. The antisense oligonucleotides may comprise phosphorothioate bonds linking between the four to six 3'-terminus nucleotides. The phosphorothioate bonds may link all the nucleotides. The phosphorothioate linkages may be mixed R_P and S_P enantiomers, or they may be stereoregular or substantially stereoregular in either R_P or S_P form.

The antisense oligonucleotides may also have sugar mimetics. The oligonucleotide may have at least one nucleotide with a modified base and/or sugar, such as a 2'-O-substituted ribonucleotide. For purposes of the invention, the term 2'-O-substituted" means substitution of the 2' position of the pentose moiety with an -O- lower alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, e.g., with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups. The oligonucleotides of the invention may include four or five ribonucleotides 2'-O- alkylated at their 5' terminus and/or four or five ribonucleotides 2'-O-alkylated at their 3' terminus.

The antisense oligonucleotides of the invention may also comprise nucleotide analogues wherein the structure of the nucleotide is fundamentally

altered. An example of such an oligonucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in DNA (or RNA) is replaced with a polyamide backbone which is similar to that found in peptides (Nielsen et al.[29]; Good and Nielsen[30]; Buchardt, deceased, et al.[31], U.S. Patent No. 5,766,855; Buchardt, deceased, et al.[32], U.S. Patent No. 5,719,262). PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind more strongly to a complementary DNA sequence than to a naturally occurring nucleic acid molecule due to the lack of charge repulsion between the PNA strand and the DNA strand.

The oligonucleotides of the present invention may also include other nucleotides comprising polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may comprise morpholino backbone structures (U.S. Patent No. 5,034,506[33]).

The oligonucleotides of the present invention are "nuclease resistant" when they have either been modified such that they are not susceptible to degradation by DNA and RNA nucleases or alternatively they have been placed in a delivery vehicle which in itself protects the oligonucleotide from DNA or RNA nucleases. Nuclease resistant oligonucleotides include, for example, methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. Suitable delivery vehicles for conferring nuclease resistance include, for example liposomes.

The oligonucleotides of the present invention may also contain groups, such as groups for improving the pharmacokinetic properties of an oligonucleotides, or groups for improving the pharmacodynamic properties of an oligonucleotide.

The antisense oligonucleotides are selected from the sequence complementary to the neuropilin gene. Preferably, the sequence exhibits the least likelihood of showing duplex formation, hair-pin formation, and homooligomer/sequence repeats but has a high to moderate potential to bind to the neuropilin gene sequences. These properties may be determined using the computer modeling program OLIGO Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN). This computer program allows the determination of a qualitative estimation of these five parameters.

Alternatively, the antisense oligonucleotides may also be selected on the basis that the sequence is highly conserved for the neuropilin gene between two or more mammalian species. These properties may be determined using the BLASTN program (Altschul, et al.[34]) of the University of Wisconsin Computer group (GCG) software (Devereux J. et al.[35]) with the National Center for Biotechnology Information (NCBI) databases.

The antisense oligonucleotides may include mutations, such as substitutions, insertions and deletions. Preferably there will be less that 10% of the sequence having mutations.

The antisense oligonucleotides generally comprise from at least about 3 nucleotides or nucleotide analogs, more preferably they are at least about 5 nucleotides, more preferably they are at least about 7 nucleotides, more preferably they are at least about 9 nucleotides and most preferably they are at least about 20 nucleotides. The antisense oligonucleotides are preferably less than about 100 nucleotides or nucleotide analogs, more preferably, less than about 50 nucleotides or nucleotide analogs, most preferably less than about 35 nucleotide or nucleotide analogs.

Preferably, the antisense oligonucleotides comprise the sequences set forth in Table 1 (below).

Table 1

Antisense oligonucleotides having a sequence complementary to the human neuropilin mRNA

SEQ ID NO.	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
1	GTI3601	GAG CGG CAG CCC CCT CTC CA	74.6	-46.5
2	GTI3602	CGA GCA CGG CGC AGA GGA GC	74.2	-45.7
3	GTI3603	GGA CGA GGG CGA GCA CGG CG	78.0	-48.6
4	GTI3604	TGG GTC CGG AGC CTG AAT CA	69.0	-42.2
5	GTI3605	TTT TTC AGG GAA TCC GGG GG	69.1	-44.6
6	GTI3606	GGG TAG TTC AGG CGG GAG CG	69.9	-44.3
7	GTI3607	AAT GGC GCC CTG TGT CCC GA	73.4	-45.4
8	GTI3608	GTG CCC AGC CAG AGC GAC TG	69.5	-42.0
9	GTI3609	TGA GGT GCG GGT GGA AGT GC	69.6	-42.0
10	GTI3610	GTG CCG ACG TGG GAC CCA GA	71.6	-43.1
11	GTI3611	GAC CCC CAG GGC ACT CAT GG	70.1	-42.9
12	GTI3612	CGA CCC CAC AGA CAG CCC CC	72.4	-44.4
13	GTI3613	TCT CTG TCC TCC AAA TCG AA	58.6	-36.5
14	GTI3614	TGC TTC CCA CCC TGA ATG AT	63.3	-39.2
15	GTI3615	TGG GAA TAG ATG AAG TTG CC	58.4	-37.1
16	GTI3617	TCC TCT GGC TTC TGG TAG CG	63.8	-39.9
17	GTI3618	AGG TTT CCT TTT CCG ATT TC	59.0	-38.6
18	GTI3619	GTG CTC CCT GTT TCA TCA AT	58.0	-36.2
19	GTI3620	CAT TGC CTG GCT TCC TGG AG	66.2	-41.1
20	GTI3621	CCC AGG GCA CTC ATG GCT AT	65.5	-41.0
21	GTI3622	GCT GAG AAA CCT TCT TTT GC	57.9	-37.0

SEQ ID NO.	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
22	GTI3623	AAC ATC TGT GGG GTT GGT GT	60.3	-36.9
23	GTI3624	TCG GAC AAA TCG AGT TAT CA	57.1	-36.0
24	GTI3625	CAA CAT TCC AGA GCA AGG AT	58.2	-36.5
25	GTI3626	CGA TCT TGA ACT TCC TCA TG	56.0	-35.2
26	GTI3627	CCT GTG AGC TGG AAG TCA TC	58.2	-35.7
27	GTI3628	CAT GTG ATA CCA GAA GGT CA	53.9	-33.5
28	GTI3629	CCA ACA GGC ACA GTA CAG CA	60.8	-36.7
29	GTI3630	ACC ATC CAC AAG TTC AAA GT	54.8	-34.5
30	GTI3631	ACC ACA GGG CTC ACC AGG CG	71.0	-43.2

The antisense oligonucleotides of Table I were selected from the sequence complementary to the human Neuropilin/$VEGF_{165}$R mRNA such that the sequence exhibits the least likelihood of showing duplex formation, hairpin formation, and homooligomers/sequence repeats but has a high potential to bind to the Neuropilin/$VEGF_{165}$R mRNA sequence. In addition, false priming to other frequently occurring or repetitive sequences in human and mouse was eliminated. These properties were determined using the computer modeling program OLIGO® Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN).

In Table 1 the "Tm" is the melting temperature of an oligonucleotide duplex calculated according to the nearest-neighbour thermodynamic values. At this temperature 50% of nucleic acid molecules are in duplex and 50% are denatured. The "ΔG" is the free energy of the oligonucleotide, which is a measurement of an oligonucleotide duplex stability.

The term "alkyl" refers to monovalent alkyl groups preferably having from 1 to 20 carbon atoms and more preferably 1 to 6 carbon atoms. This term is

exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and the like.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is either fluoro or chloro.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The material is compatible with a biological system such as a cell, cell culture, tissue or organism.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the antisense oligonucleotides of this invention and which are not biologically or otherwise undesirable. In many cases, the antisense oligonucleotides of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethylamine, diethylamine, tri(*iso*-propyl)amine, tri(*n*-propyl)amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice

of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "neuropilin gene" refers to any gene which encodes a protein that is capable of acting as a receptor for semaphorin or VEGF. Preferably, the neuropilin mRNA has a sequence substantially similar to that shown in Figures 5, 6 or 7.

The term "complementary to" means that the antisense oligonucleotide sequence is capable of binding to the target sequence, i.e. the neuropilin gene (or mRNA). Preferably, the antisense oligonucleotide binds to the nucleic acid sequence under physiological conditions, e.g. by Watson-Crick base pairing (interaction between oligonucleotide and single-stranded nucleic acid) or by Hoogsteen base pairing (interaction between oligonucleotide and double-stranded nucleic acid) or by any other means including in the case of an oligonucleotide binding to RNA, causing pseudoknot formation. Binding by Watson-Crick or Hoogsteen base pairing under physiological conditions is measured as a practical matter by observing interference with the function of the nucleic acid sequence.

Preferably the antisense oligonucleotide sequence has at least about 75% identity with the target sequence, preferably at least about 90% identity and most preferably at least about 95% identity with the target sequence allowing for gaps or mismatches of several bases. Identity can be determined, for example, by using the BLASTN program of the University of Wisconsin Computer Group (GCG) software. Preferably the antisense oligonucleotide sequence hybridizes to the neuropilin mRNA with a melting temperature of at least 45°C, more preferably at least about 50°C and most preferably at least about 55°C as determined by the OLIGO primer analysis software program version 5.0 described herein.

The term "inhibiting growth" means a reduction or inhibition in the growth of at least one tumor cell type by at least 10%, more preferably of at least 50% and most preferably of at least 75%. The reduction in growth can be determined for tumor cells by measuring the size of the tumor in nude mice or the inability of the tumor cells to form colonies *in vitro*.

The term "inhibiting angiogenesis" means a reduction or inhibition in neovascularization. This can be determined by methods known in the art. A murine model of oxygen-induced retinal neovascularization has been established which occurs in 100% of treated animals and is quantifiable (45, 46). Using this model, a correlation between the inhibition of neuropilin and inhibition of retinal neovascularization could be measured. This result may also be confirmed by changes in expression level of neuropilin by Northern blot and in situ hybridization analysis.

The term "inhibiting metastasis" means reducing or inhibiting the number of metastatic tumors that develop, preferably by at least 10%, more preferably by at least 50%. This can be determined by the methods set forth in the Examples and other methods known in the art.

The term "inhibiting expression of neuropilin" means that the antisense oligonucleotide reduces the level of neuropilin mRNA or the level of neuropilin protein produced by the cell when the oligonucleotide is administered to the cell.

The term "mammal" or "mammalian" means all mammals including humans, ovines, bovines, equines, swine, canines, felines and mice, etc., preferably it means humans.

A "mammal suspected of having a tumor" means that the mammal may have a proliferative disorder or tumor or has been diagnosed with a proliferative disorder or tumor or has been previously diagnosed with a proliferative disorder or tumor, the tumor has been surgically removed and the mammal is suspected of harboring some residual tumor cells.

Preparation of the Antisense Oligonucleotides

The antisense oligonucleotides of the present invention may be prepared by conventional and well-known techniques. For example, the oligonucleotides may be prepared using solid-phase synthesis and in particular using commercially available equipment such as the equipment available from Applied Biosystems Canada Inc., Mississauga, Canada. The oligonucleotides may also be prepared by enzymatic digestion of the naturally occurring neuropilin gene by methods known in the art.

These oligonucleotides can be prepared by the art recognized methods such as phosphoramidate or H-phosphoate chemistry which can be carried out manually or by an automated synthesizer as described by Uhlmann et al.(43) and Agrawal et al.(44)

Isolation and Purification of the Antisense Oligonucleotides

Isolation and purification of the antisense oligonucleotides described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. However, other equivalent separation or isolation procedures could, of course, also be used.

An expression vector comprising the antisense oligonucleotide sequence may be constructed having regard to the sequence of the oligonucleotide and using procedures known in the art.

Vectors can be constructed by those skilled in the art to contain all the expression elements required to achieve the desired transcription of the antisense oligonucleotide sequences. Therefore, the invention provides vectors comprising a transcription control sequence operatively linked to a sequence which encodes an antisense oligonucleotide. Suitable transcription and translation elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes. Selection of appropriate elements is dependent on the host cell chosen.

Reporter genes may be included in the vector. Suitable reporter genes include β-galactosidase (e.g. lacZ), chloramphenicol, acetyl-transferase, firefly luciferase, or an immunoglobulin or portion thereof. Transcription of the antisense oligonucleotide may be monitored by monitoring for the expression of the reporter gene.

The vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al.[24]; Ausubel et al.[25]; Chang et al.[36]; Vega et al.[37]; and Vectors: A Survey of Molecular Cloning Vectors and Their Uses[38] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

Introduction of nucleic acids by infection offers several advantages. Higher efficiency and specificity for tissue type can be obtained. Viruses typically infect and propagate in specific cell types. Thus, the virus' specificity may be used to target the vector to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

It is contemplated that the oligonucleotide of this invention may be a ribozyme which cleaves the mRNA. The ribozyme preferably has a sequence homologous to a sequence of an oligonucleotide of the invention and the necessary catalytic center for cleaving the mRNA. For example, a homologous ribozyme sequence may be selected which destroys the neuropilin mRNA. The ribozyme type utilized in the present invention may be selected from types known in the art. Several ribozyme structural families have been identified including Group I introns, RNase P, the hepatitis delta virus ribozyme, hammerhead ribozymes and the hairpin ribozyme originally derived from the negative strand of the tobacco ringspot virus satellite RNA (sTRSV) (Sullivan 1994, U.S. Patent No. 5,225,347[39]). Hammerhead and hairpin ribozyme motifs are most commonly adapted for trans cleavage of mRNAs for gene therapy (Sullivan 1994). Hairpin ribozymes are preferably used in the present invention. In general, the ribozyme is from 30 to 100 nucleotides in length.

The oligonucleotides of the invention may be insolubilized. For example, the oligonucleotide may be bound to a suitable carrier. Examples of suitable carriers are agarose, cellulose, dextran, Sephadex, Sepharose, carboxymethyl cellulose polystyrene, filter paper, ion-exchange resin, plastic film, plastic tube, glass beads, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk etc. The carrier may in the shape of, for example, a tube, test plate, beads disc, sphere etc.

The insoubilized oligonucleotide may be prepared by reacting the material with the suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling.

Pharmaceutical Formulations

When employed as pharmaceuticals, the antisense oligonucleotides are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound. The pharmaceutical composition is, for example, administered intravenously. It is contemplated that the pharmaceutical composition may be administered directly into the tumor to be treated.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the antisense oligonucleotides associated with pharmaceutically acceptable carriers or excipients. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent,

it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 1% to about 95%, more usually about 5% to about 90% of the active ingredient. The term "unit dosage forms" refers to physically

discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The antisense oligonucleotide is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. An effective amount is that amount which when administered alleviates the symptoms. Preferably the effective amount is that amount able to inhibit tumor cell growth. Preferably the effective amount is from about 0.1 mg/kg body weight to about 20 mg/kg body weight. It will be understood, however, that the amount of the antisense oligonucleotide actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like. The course of therapy may last from several days to several months or until diminution of the disease is achieved. The antisense oligonucleotide may be administered in combination with other known therapies. When co-administered with one or more other therapies, the oligonucleotide may be administered either simultaneously with the other treatments(s), or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the oligonucleotide in combination with the other therapy.

For preparing solid compositions such as tablets, the principal active ingredient/antisense oligonucleotide is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed

evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described herein. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a

face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The pharmaceutical composition of the invention may be in the form of a liposome, in which the oligonucleotide is combined, in addition to other pharmacuetically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micells, insoluble monolayers, liquid crystals or lamellar layers which are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids and the like. One particularly useful lipid carrier is lipofectin. Preparation of such liposomal formulations is within the skill in the art, for example, International Patent No. WO97/21808 (28) The pharmaceutical composition may further include compounds such as cyclodextrins and the like which enhance delivery of oligonucleotides into cells or slow release polymers.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the antisense oligonucleotides of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, for example, U.S. Patent 5,023,252[40], herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Another preferred method of delivery involves "shotgun" delivery of the naked antisense oligonucleotides across the dermal layer. The delivery of "naked" antisense oligonucleotides is well known in the art. See, for example, Felgner et

al.,U.S. Patent No. 5,580,859[41]. It is contemplated that the antisense oligonucleotides may be packaged in a lipid vesicle before "shotgun" delivery of the antisense oligonucleotide.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg

Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	5.0 mg
Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences*[23].

The antisense oligonucleotides or the pharmaceutical composition comprising the antisense oligonucleotides may be packaged into convenient kits providing the necessary materials packaged into suitable containers.

The antisense oligonucleotides of the invention in the form of a therapeutic formulation are useful in treating diseases, and disorders and conditions associated with angiogenesis and neovascularization including, but not limited to, retinal neovascularization and tumor growth. In such methods a therapeutic amount of a oligonucleotide effective in inhibiting the expression of neuropilin is administered to a cell. This cell may be part of a cell culture, a tissue culture, or the whole body of a mammal such as a human.

The oligonucleotides and ribozymes of the invention modulate tumor cell growth. Therefore methods are provided for interfering or inhibiting tumor cell growth in a mammal comprising contacting the tumor or tumor cells with an

antisense oligonucleotide of the present invention. Without being limited to a theory or mechanism, it is believed that the antisense oligonucleotides may inhibit tumor growth in two ways. They may inhibit growth in an autocrine manner by acting directly on the tumor cells. Alternatively or additionally, the antisense oligonucleotides may act by inhibiting neovascularization associated with tumor growth, thereby reducing the blood supply available to the tumor.

The term "contact" refers to the addition of an oligonucleotide, ribozyme, etc. to a cell suspension or tissue sample or administering the oligonucleotides etc. directly or indirectly to cells or tissues within an animal.

The methods may be used to treat proliferative disorders including various forms of cancer or tumors such as sarcomas, melanomas, adenomas, carcinomas of solid tissue, hypoxic tumors, squamous cell carcinomas of the mouth, throat, larynx and lung, genitourinary cancers such as cervical and bladder cancer, hematopoietic cancers, colon cancer, breast cancer, pancreatic cancer, renal cancer, brain cancer, skin cancer, liver cancer, head and neck cancers, and nervous system cancers, as well as benign lesions such as papillomas.

The methods may be use to treat neovascular disorders such as diabetic retinopathy, retinopathy of prematurity and age related macular degeneration.

The oligonucleotides of the invention may also be used to treat drug resistant tumors. Examples of drug resistant tumors are tumors resistant to such chemotherapeutic agents as 5-fluorouracil, mitomycin C, methotrexate or hydroxyurea and tumors expressing high levels of P-glycoprotein which is known to confer resistance to multiple anticancer drugs such as colchicine, vinblastine and doxorubicin; or tumors expressing multi-drug resistance protein as described by Dreeley et al.[42]. Accordingly, it is contemplated that the oligonucleotides of the

present invention may be administered in conjunction with or in addition to known anticancer compounds or chemotherapeutic agents. Chemotherapeutic agents are compounds which may inhibit the growth of tumors. Such agents, include, but are not limited to, 5-fluorouracil, mitomycin C, methotrexate and hydroxyurea. It is contemplated that the amount of chemotherapeutic agent administered may be either an effective amount, i.e. an amount sufficient to inhibit tumor growth or a less than effective amount.

The oligonucleotides of the present invention have been found to reduce the growth of tumors that are metastatic such as MDA-MB-231 breast adenocarcinoma, HT-29 colon adenocarcinoma, A549 lung carcinoma, and A2058 melanoma cancer cells. In an embodiment of the invention, a method is provided for reducing the growth of metastastic tumors in a mammal comprising administering an amount of an oligonucleotide complementary to the neuropilin mRNA, or an oligonucleotide shown in Table 1.

The oligonucleotides of the present invention may reduce angiogenesis. In one embodiment of the invention a method is provided for the treatment of neovascular disorders.

The oligonucleotides may be delivered using viral or non-viral vectors. Sequences may be incorporated into cassettes or constructs such that an oligonucleotide of the invention is expressed in a cell. Preferably, the construct contains the proper transcriptional control region to allow the oligonucleotide to be transcribed in the cell.

Therefore, the invention provides vectors comprising a transcription control sequence operatively linked to a sequence which encodes an oligonucleotide of the

invention. The present invention further provides host cells, selected from suitable eucaryotic and procaryotic cells, which are transformed with these vectors.

Suitable vectors are known and preferably contain all of the expression elements necessary to achieve the desired transcription of the sequences. Phagemids are a specific example of such beneficial vectors because they can be used either as plasmids or as bacteriophage vectors. Examples of the vectors include viruses such as bacteriophages, baculoviruses, retroviruses, DNA viruses, liposomes and other recombination vectors. The vectors can also contain elements for use in either procaryotic or eucaryotic host systems. One of ordinary skill in the art will know which host systems are compatible with a particular vector.

The vectors can be introduced into the cells by stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with recombinant viruses. An example of such a negative selection marker is the TK gene which confers sensitivity to the antiviral gancyclovir. Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

Retroviral vectors are another example of vectors useful for the *in vivo* introduction of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is the process by which a single infected cell produces many progeny virions that infect neighboring cells. The result is that a large area becomes rapidly infected.

A vector to be used in the methods of the invention may be selected depending on the desired cell type to be targeted. For example, if breast cancer is to be treated, then a vector specific for epithelial cells may be used. Similarly, if cells of the hematopoietic system are to be treated, then a viral vector that is specific for blood cells is preferred.

Utility

The antisense oligonucleotides of the present invention may be used for a variety of purposes. They may be used to inhibit the expression of the neuropilin gene in a mammalian cell, resulting in the inhibition of growth of that cell. They may be used to inhibit tumor cell growth and/or neovascularization. The oligonucleotides may be used as hybridization probes to detect the presence of the neuropilin mRNA in mammalian cells. When so used the oligonucleotides may be labeled with a suitable detectable group (such as a radioisotope, a ligand, another member of a specific binding pair, for example, biotin). Finally, the oligonucleotides may be used as molecular weight markers.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celsius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated).

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning:

AS	=	antisense
cDNA	=	complementary deoxyribonucleic acid
ODN	=	oligonucleotide
μM	=	micromolar
mM	=	millimolar
M	=	molar
ml	=	milliliter
μl	=	microliter
mg	=	milligram
μg	=	microgram
PAGE	=	polyacrylamide gel electrophoresis
rpm	=	revolutions per minute
ΔG	=	free energy, a measurement of oligonucleotide duplex stability
kcal	=	kilocalories
FBS	=	fetal bovine serum
DTT	=	dithiothrietol
SDS	=	sodium dodecyl sulfate
PBS	=	phosphate buffered saline
PMSF	=	phenylmethylsulfonyl fluoride
GAPDH	=	glyceraldehyde-3-phosphate dehydrogenase
IgG	=	immunoglobulin G
kDa	=	kilodalton
PCR	=	polymerase chain reaction
Tris-HCl	=	Tris(hydroxymethyl)aminomethane-hydrochloric acid
TRIzol	=	total RNA isolation reagent
VEGF	=	vascular endothelial growth factor

General Methods in Molecular Biology:

Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al.[24]; Ausubel et al.[25]; and Perbal[26].

Oligonucleotides

The antisense oligonucleotides were selected from the sequence complementary to the neuropilin mRNA such that the sequence exhibits the least likelihood of showing duplex formation, hairpin formation, and homooligomers/sequence repeats but has a high potential to bind to the neuropilin mRNA sequence. In addition, a false priming to other frequently occurring or repetitive sequences in human and mouse was eliminated. These properties were determined using the computer modeling program OLIGO® Primer Analysis Software, Version 5.0 International Biosciences, Inc. Plymouth MN). Based on this analysis, phosphorothioate antisense oligonucleotides were designed and then made by methods well known in the art.

Cell Lines

Seven different human cancer cell lines including lung carcinoma (A549), melanoma (C8161), breast cell adenocarcinoma (MDA-MB-231), metastatic pancreatic adenocarcinoma (AsPC-1), colon adenocarcinoma (HT-29), human melanoma cell line A2058, human pancreatic cancer PC3 were obtained from American Type Culture Collection (ATCC). The cell lines were maintained in α-

MEM medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS).

Example 1. The inhibition of growth of cancer cell lines by antisense oligonucleotides complementary to neuropilin

The colony forming ability of cancer cell lines treated with different antisense oligonucleotides was estimated using a method previously described (Choy et al.[18]). Specifically, aliquots of a tumor cell suspension were seeded into 60 mm tissue culture dishes at a density of approximately $1X10^4$ and incubated overnight at 37°C in α-MEM medium supplemented with 10% FBS. Cells were washed once in 5 ml of PBS and treated with 0.2 μM of the indicated antisense oligonucleotides in the presence of cationic lipid (Lipofectin reagent, final concentration, 5 μg/ml, Gibco-BRL, Gaithersburg, MD) for 4 hours. The antisense oligonucleotides were removed by washing the cells once with PBS and the cells were cultured in growth medium (α-MEM medium supplemented with 10% FBS) for 7 to 10 days at 37°C. Colonies were stained with methylene blue and scored by direct counting as described (Choy et al.[18] and Huang and Wright[20]). Percent inhibition was calculated by comparison with the number of colonies present in cultures grown in the absence of antisense oligonucleotides. All experiments were performed in quadruplicate.

The antisense oligonucleotides exerted inhibitory effects on the colony forming ability of the human tumor cell lines. The percent inhibition of each antisense oligonucleotide is shown in Fig. 1A for human melanoma cell line C8161; Fig. 1B for human lung cancer cell line A549; Fig. 1C for human melanoma cell line A2058: Fig. 1D for human colon cancer cell line HT-29; Fig. 1E for human prostate cancer cell line PC-3; and Fig 1F for human pancreatic cancer cell line AsPC-1.

Example 2 Decreased mRNA levels following treatment with antisense oligonucleotides complementary to neuropilin

Human melanoma cancer cells (A2058) or breast cancer cells (MDA-MB-231) were grown to subconfluency (70-80%) and were treated with 0.2 μM of phosphorothioate antisense oligonucleotides complementary to neuropilin for 4 hours in the presence of cationic lipid (Lipofectin reagent, final concentration, 5 μg/ml, Gibco-BRL) and Opti-MEM (Gibco-BRL). Cells were washed once with PBS and incubated for 16 hours in α-MEM medium (Gibco-BRL) containing 10% FBS. Total RNA was prepared in TRIzol reagent (Gibco-BRL) and Northern blot analysis was performed as described in Hurta and Wright(27) with some modifications. The bolts were hybridized with ^{32}P-labeled 598 bp PCR fragments synthesized using forward primer (5'-CGC TCC CGC CTG AAC TAC CC-3') [SEQ ID NO:31], reverse primier (5'-TCC CAC CCT GAA TGA TGA TG-3') [SEQ ID NO:32] and the human colorectal adenocarcinoma 5'-stretch plus cDNA library (Clonetech, Palo Alto CA) as a template. Human neuropilin/VEGF$_{165}$R mRNA was expressed as a ~ 7 kb nucleotide transcript (Soker et al.[9]). Equal RNA loading was demonstrated by methylene blue staining of the blot prior to hybridization.

Fig. 2A and 2B show that the antisense oligonucleotides reduce the neuropilin mRNA levels to at least 50% of the control cells.

Example 3. Inhibition of human tumor cell growth in mice by intravenous treatment with antisense oligonucleotides complementary to neuropilin

CD-1 athymic nude mice were purchased from Charles River Laboratories (Montreal Canada). HT-29 human colon cancer cells (typically 3X10^6 cells in 100 μl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old CD-1

athymic female nude mice. Each experimental group included 5 mice. After the size of tumor reached an approximate volume of 100 mm^3, typically 5 days post tumor cell injection, the antisense oligonucleotide GTI3602 [SEQ ID NO:2] was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Treatments typically lasted 14 days thereafter.

Fig. 3A shows the effects of the antisense oligonucleotide GTI3602 on HT-29 tumor growth in CD-1 nude mice. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with a caliper on average of two day intervals over the span of 14 days. Each point in the figure represents mean tumor volume calculated from 5 animals per experimental group. Analysis of covariance was used to compare the regression curves of mice over time within each treatment group. Specific hypothesis of equality of slopes, or equality of intercepts when slopes are equal are derived from the analysis. All analysis used the SAS (Statistical Analysis System) version 6.12. When compared to the saline control, administration of the antisense oligonucleotide inhibited the growth of the tumor with a p value of ≤ 0.0001.

At the end of the treatment (usually 24 hours after the last treatment) the animals were sacrificed and tumor weights were measured. Fig. 3B shows the mean weight of the tumors. The antisense oligonucleotide showed significant inhibitory effects on tumor growth. One-way analysis of variance was used to compare the means of groups of treatments. Where the overall group effect was significant, *a priori* multiple comparisons using the least square means was used to find the pairs of treatment groups that were significantly different. When tumor weight was compared the antisense oligonucleotide also showed statistically significant inhibition when compared to the saline control.

Example 4. Inhibition of Experimental Metastasis by Antisense Oligonucleotides

Experimental metastasis of C8161 human melanoma cells treated with different antisense oligonucleotides was estimated as previously described (Fan et al., 1996[19]). Aliquots of cell suspension were seeded into 100 mm tissue culture dishes at a density of 2 X 10^6 and incubated overnight at 37°C in α-MEM medium supplemented with 10% FBS. Cells were washed once in 10 ml of PBS and treated with 0.2 μM of oligonucleotides in the presence of cationic lipid (Lipofectin reagent, final concentration, 5 μg/ml, Gibco-BRL) for 4 hours. The antisense oligonucleotides were removed by washing the cells once with PBS and the cells were trypsinized. Cells were then collected by centrifugation, and approximately 1 X 10^5 cells suspended in 0.1 ml of PBS were injected into the tail veins of 6- 8 week old CD-1 athymic female nude mice. Estimates of the number of lung tumors were made 5 weeks later, after excised lungs from individual mice were stained with picric acid dye solution (75% picric acid, 20 % formaldehyde, 5% glacial acetic acid).

Fig. 4 shows the reduced number of lung tumors in the female nude mice after treatment of the tumor cells with various antisense oligonucleotides.

Claims:

1. An antisense oligonucleotide from about 20 to about 100 nucleotides comprising a sequence selected from the group consisting of SEQ ID NOs:1 - 30 as set forth in Table 1 which oligonucleotide inhibits neuropilin expression.

2. The antisense oligonucleotide of Claim 1 further comprising one or more phosphorothioate internucleotide linkages

3. The antisense oligonucleotide of Claim 1 further comprising additional nucleotides not complementary to the neuropilin mRNA.

4. A vector comprising an oligonucleotide sequence from about 20 to 100 nucleotides comprising a sequence selected from the group consisting of SEQ ID NOs:1 - 30 as set forth in Table 1 which oligonucleotide inhibits neuropilin expression.

5. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of the antisense oligonucleotide from about 20 to 100 nucleotides comprising a sequence selected from the group consisting of SEQ ID NOs:1 - 30 as set forth in Table 1 which inhibit neuropilin expression.

6. A method for inhibiting the growth of a mammalian tumor comprising, administering to a mammal suspected of having the tumor an effective amount of an antisense oligonucleotide from about 3 to about 100 nucleotides comprising a sequence complementary to a mammalian neuropilin mRNA under conditions such that the growth of the tumor is inhibited.

7. The method according to Claim 6 further comprising the step of administering to the mammal a chemotherapeutic agent.

8. The method according to Claim 6 wherein the oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NOs:1 - 30 as set forth in Table 1.

9. The method according to Claim 6 wherein the oligonucleotide is nuclease resistant.

10. A method for inhibiting the metastasis of a mammalian tumor comprising, administering to a mammal suspected of having a metastatic tumor an effective amount of an antisense oligonucleotide from about 3 nucleotides to about 100 nucleotides comprising a sequence complementary to a mammalian neuropilin gene under conditions such that the metastasis of the tumor is inhibited.

11. The method according to Claim 10 further comprising the step of administering to the mammal a chemotherapeutic agent.

12. The method according to Claim 10 wherein the oligonucleotide is nuclease resistant.

13. The method according to Claim 10 wherein the oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NOs:1 - 30 as set forth in Table 1.

14. A method for inhibiting neovascularization comprising, administering to a mammal an effective amount of an antisense oligonucleotide from about 3 nucleotides to about 100 nucleotides comprising a sequence complementary to a

mammalian neuropilin gene under conditions such that neovascularization is inhibited.

15. The method according to Claim 14 wherein the oligonucleotide is nuclease resistant.

16. The method according to Claim 14 wherein the oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NOs:1 - 30 as set forth in Table 1.

ABSTRACT

This invention relates to oligonucleotides complementary to the neuropilin genes which modulate tumor cell growth and angiogenesis in mammals. This invention is also related to methods of using such compounds in inhibiting the growth of tumor cells and angiogenesis in mammals. This invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

Inhibition of Human Melanoma C8161 Colony Forming Ability by 12 Different Antisense ODNs

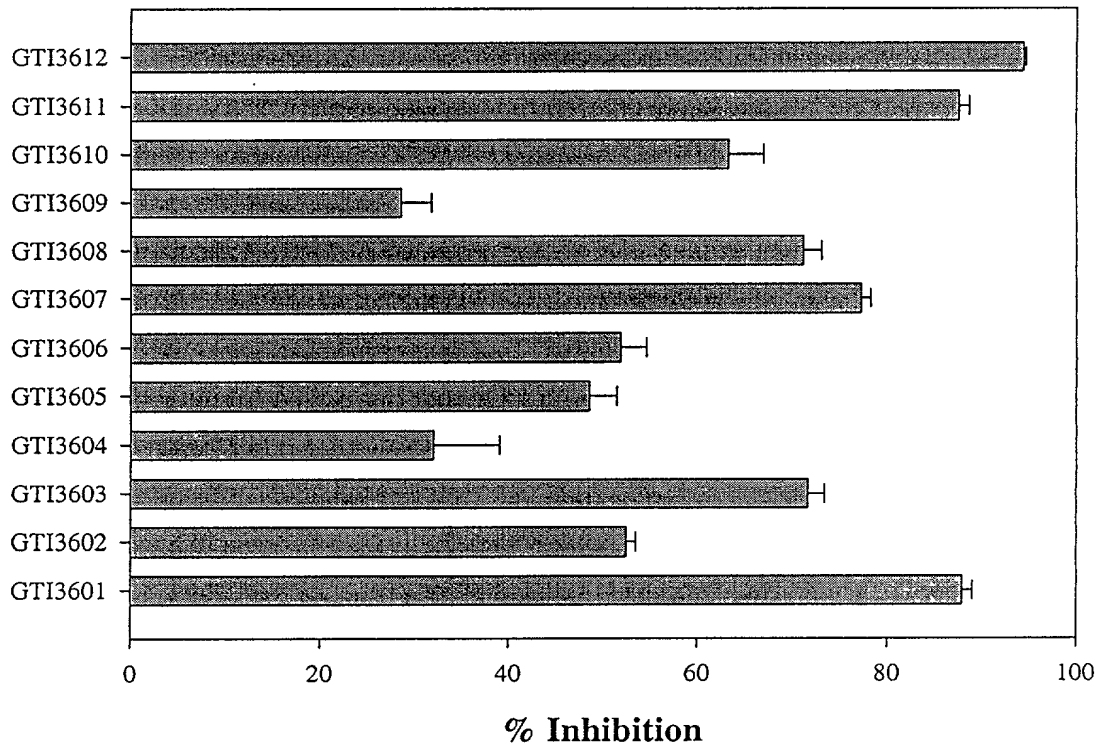

Fig. 1A

Inhibition of Human Lung Cancer A549 Colony Forming Ability by 12 Different Antisense ODNs

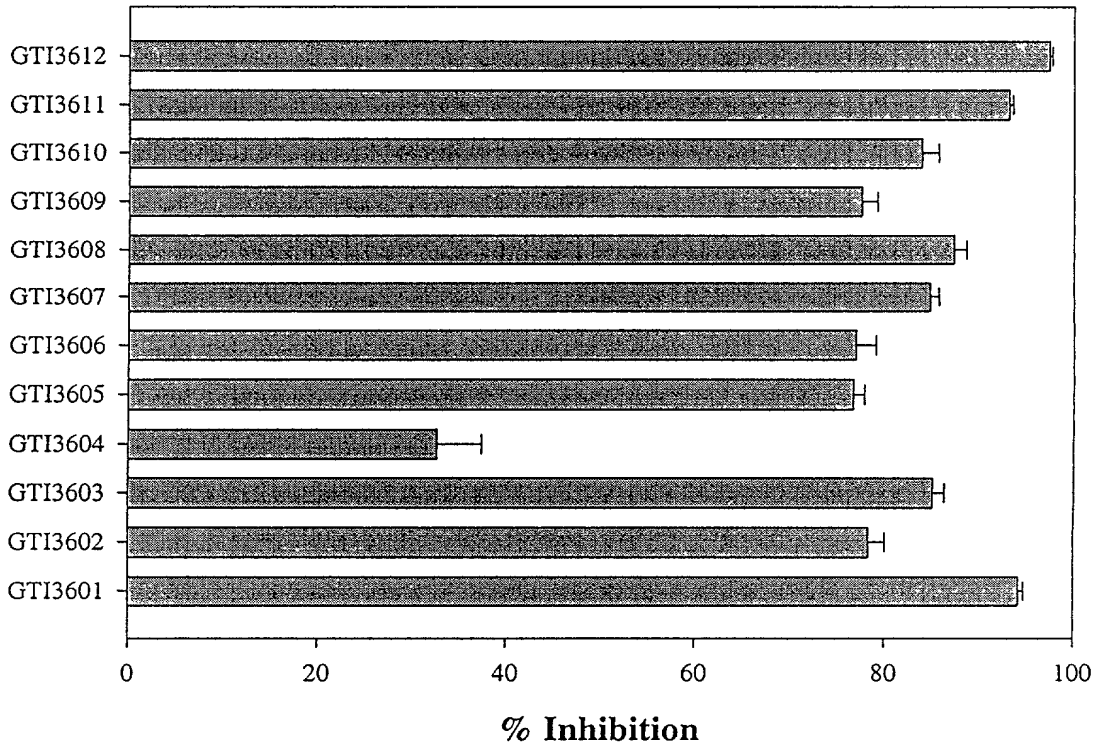

Fig. 1B

Inhibition of Human melanoma A2058 Colony Forming Ability by 12 Different Antisense ODNs

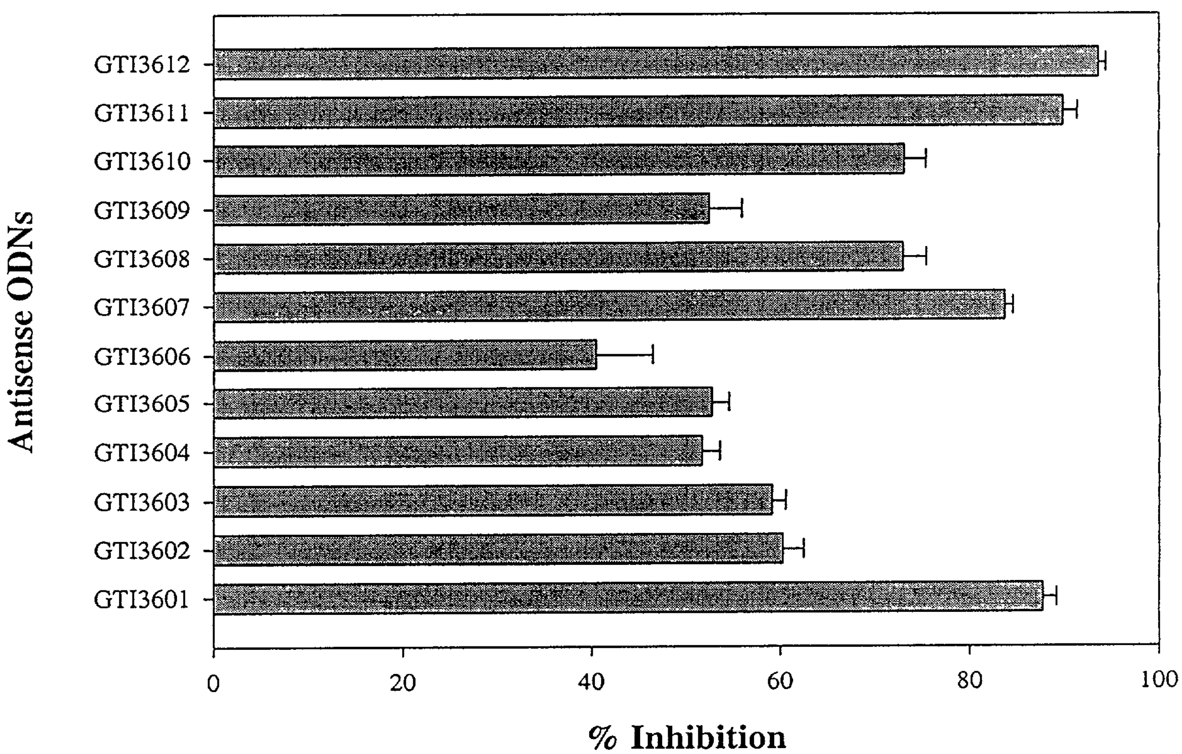

Fig. 1C

Inhibition of Human Colon Cancer HT-29 Colony Forming Ability by 12 Different Antisense ODNs

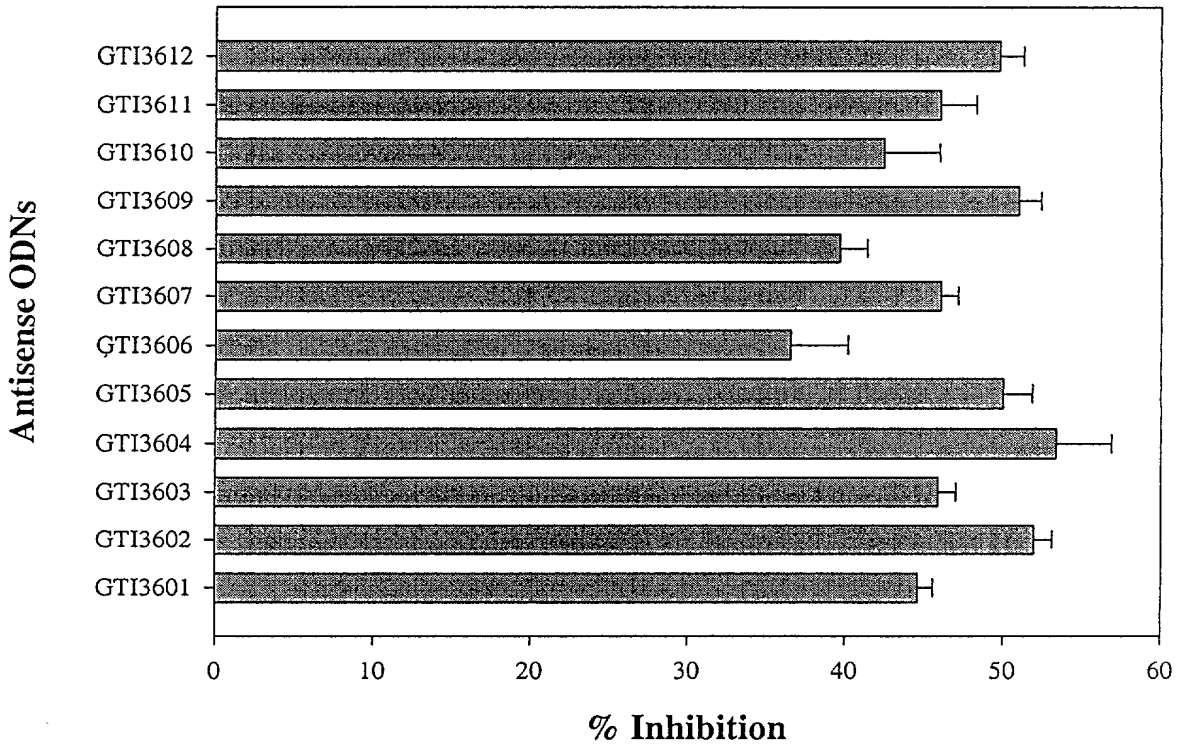

Fig. 1D

Inhibition of Human Prostate Cancer PC-3 Colony Forming Ability by 12 Different Antisense ODNs

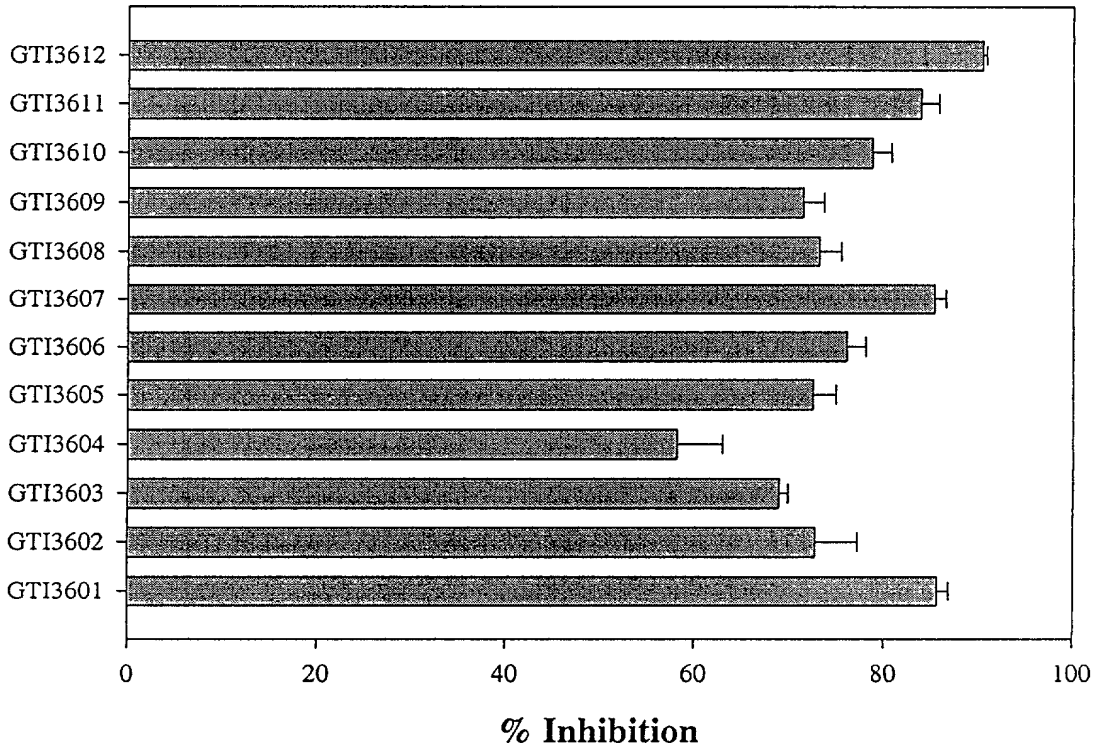

Fig. 1E

Inhibition of Human Pancreatic Cancer AsPC-1 Colony Forming Ability by 12 Different Antisense ODNs

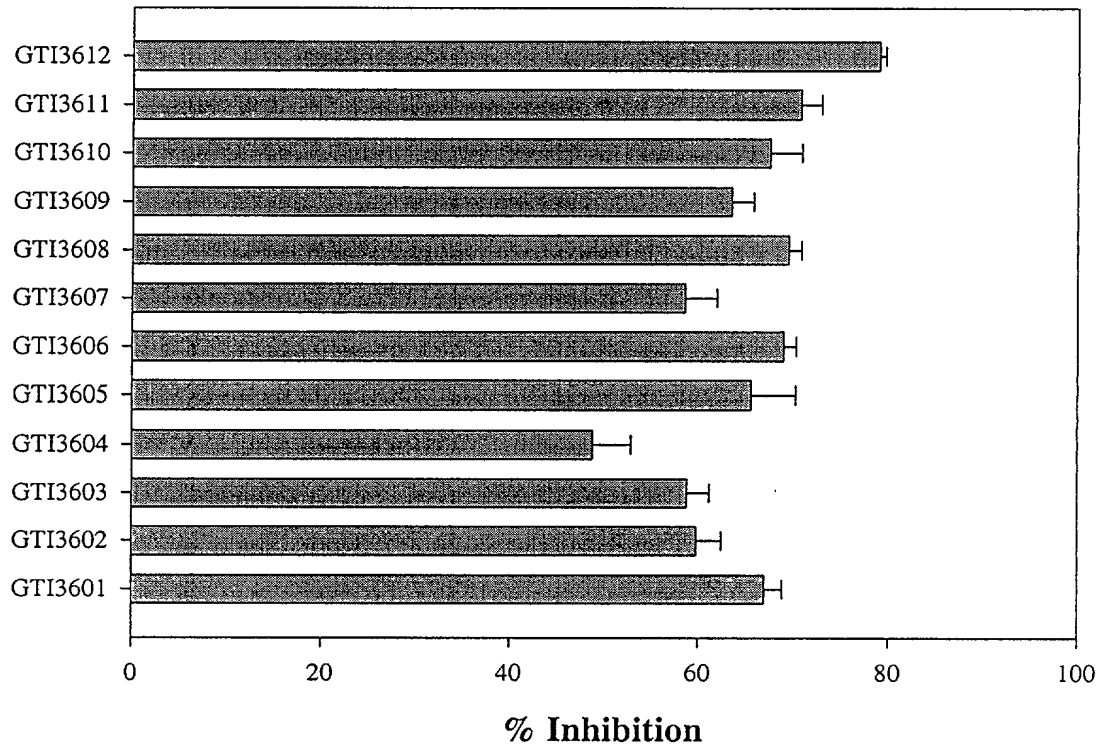

Fig. 1F

Examples of Decreased mRNA Levels following Treatment with Antisense ODNs

Breast Cancer Cells (MDA-MB-231)

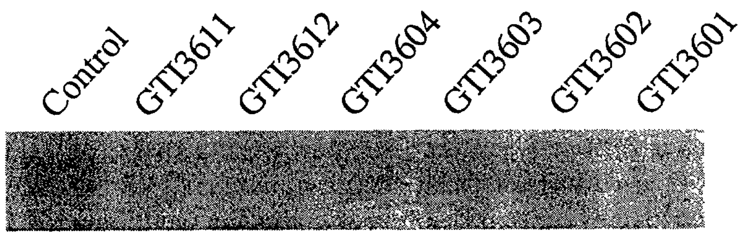

Fig. 2A

Melanoma Cells (A2058)

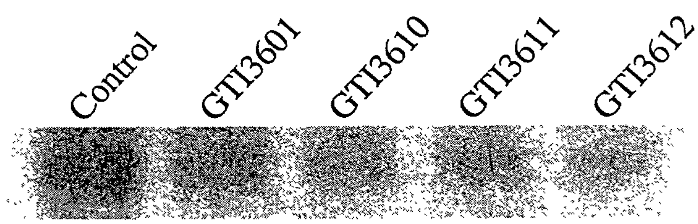

Fig. 2B

Effects of GTI3602 Antisense ODN treatment on Human Tumor Growth in Mice

a

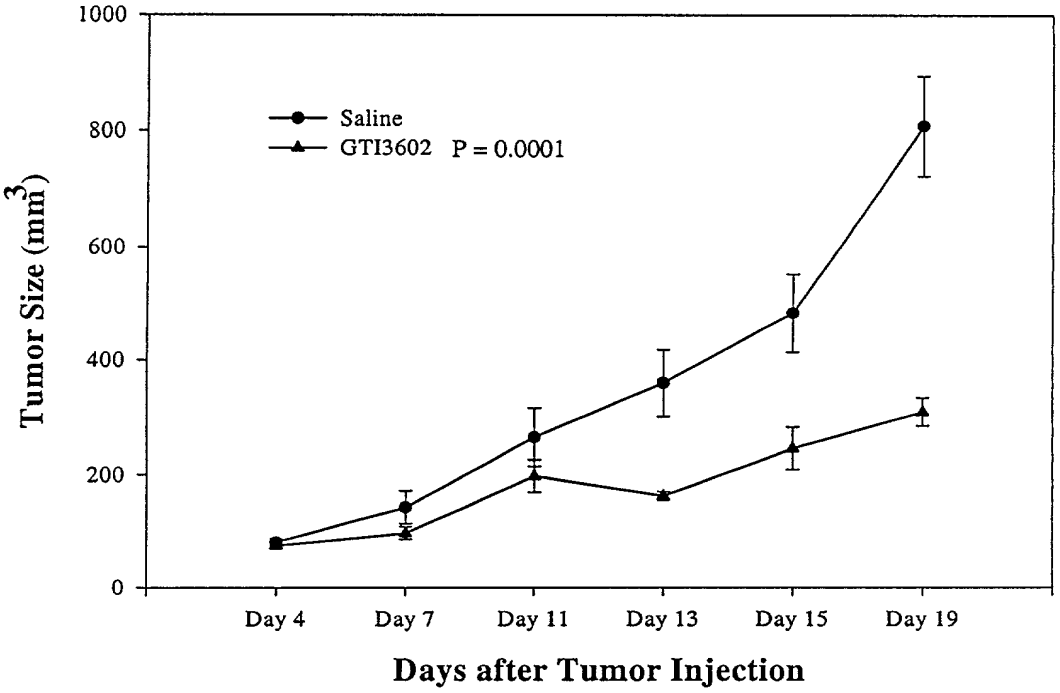

Fig. 3A

b

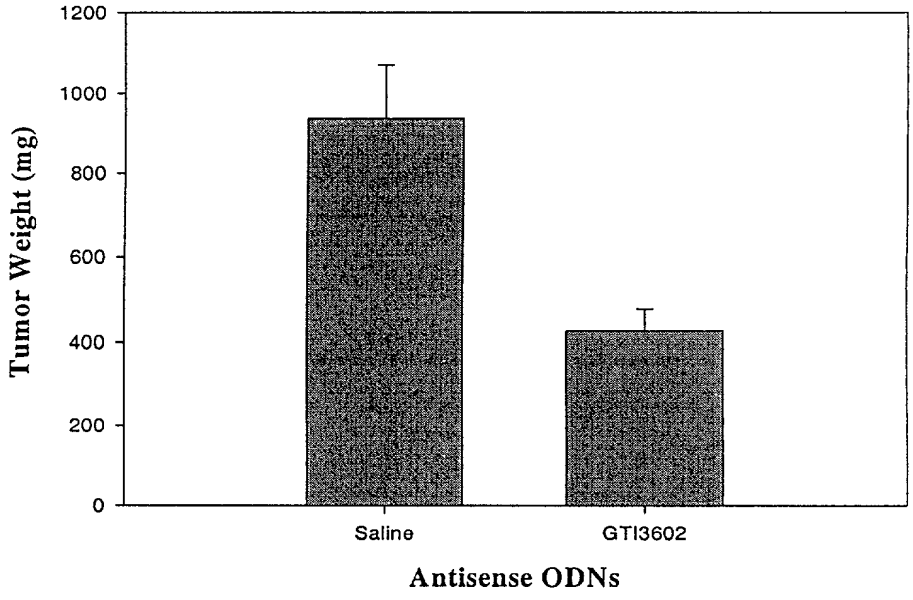

Fig. 3B

Reduction of Tumor Metastases

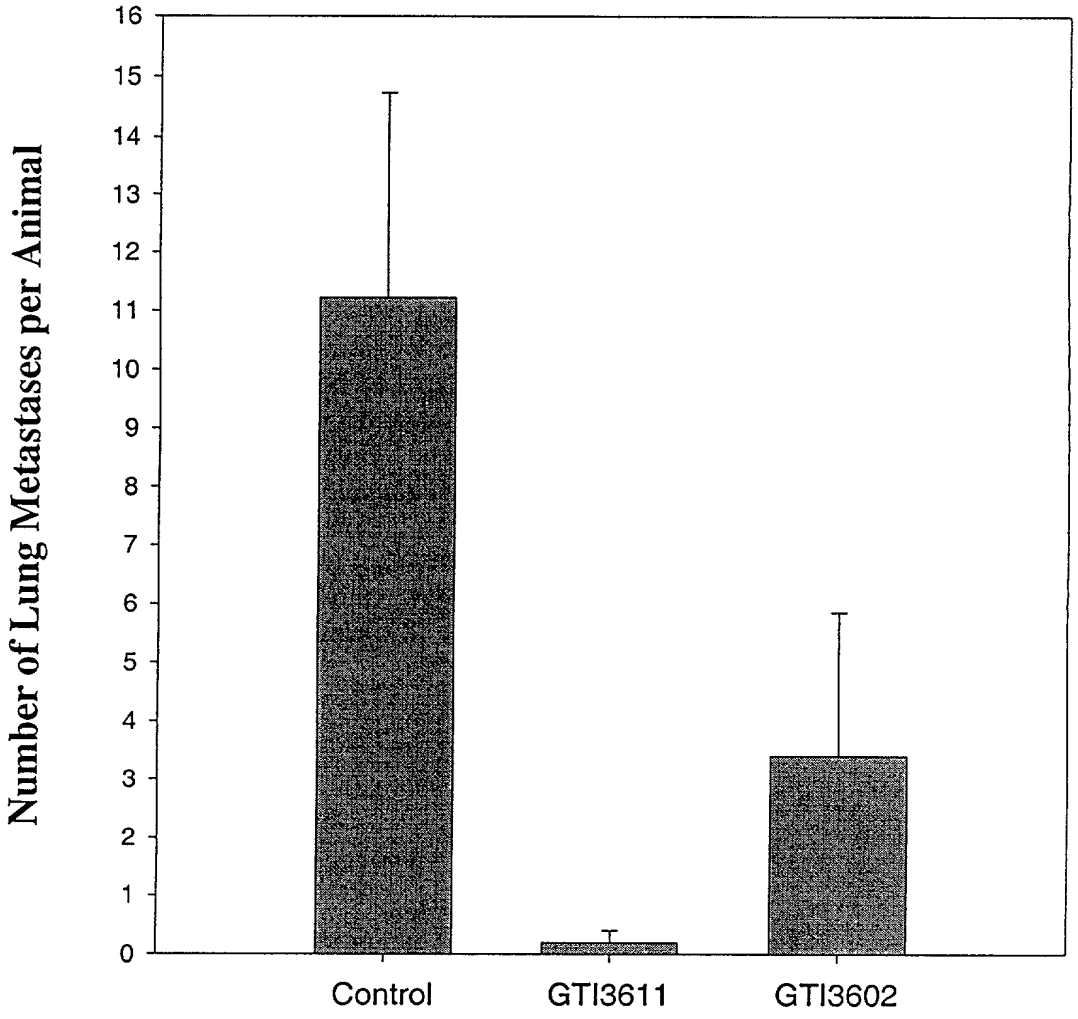

Fig. 4

```
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CCGGACCCAT ACCAGAGAAT TATGATCAAC TTCAACCCTC ACTTCGATTT GGAGGACAGA     240
GACTGCAAGT ATGACTACGT GGAAGTCTTC GATGGAGAAA ATGAAAATGG ACATTTTAGG     300
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```

Fig. 5

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```

Fig. 6

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ACCCTGAGAG	AGCCACACAC	AGTGGGCTTG	GGCTGAGGAT	GGAGCTACTG	GGCTGTGAAG	2100
TGGAAGCACC	TACAGCTGGA	CCAACCACAC	CCAATGGGAA	CCCAGTGCAT	GAGTGTGACG	2160
ACGACCAGGC	CAACTGCCAC	AGTGGCACAG	GTGATGACTT	CCAGCTCACA	GGAGGCACCA	2220
CTGTCCTGGC	CACAGAGAAG	CCAACCATTA	TAGACAGCAC	CATCCAATCA	GAGTTCCCGA	2280
CATACGGTTT	TAACTGCGAG	TTTGGCTGGG	GCTCTCACAA	GACATTCTGC	CACTGGGAGC	2340
ATGACAGCCA	TGCACAGCTC	AGGTGGAGTG	TGCTGACCAG	CAAGACAGGG	CCGATTCAGG	2400
ACCATACAGG	AGATGGCAAC	TTCATCTATT	CCCAAGCTGA	TGAAAATCAG	AAAGGCAAAG	2460
TAGCCCGCCT	GGTGAGCCCT	GTGGTCTATT	CCCAGAGCTC	TGCCCACTGT	ATGACCTTCT	2520
GGTATCACAT	GTCCGGCTCT	CATGTGGGTA	CACTGAGGGT	CAAACTACGC	TACCAGAAGC	2580
CAGAGGAATA	TGATCAACTG	GTCTGGATGG	TGGTTGGGCA	CCAAGGAGAC	CACTGGAAAG	2640
AAGGACGTGT	CTTGCTGCAC	AAATCTCTGA	AACTATATCA	GGTTATTTTT	GAAGGTGAAA	2700
TCGGAAAAGG	AAACCTTGGT	GGAATTGCTG	TGGATGATAT	CAGTATTAAC	AACCATATTT	2760
CTCAGGAAGA	CTGTGCAAAA	CCAACAGACC	TAGATAAAAA	GAACACAGAA	ATTAAAATTG	2820
ATGAAACAGG	GAGCACTCCA	GGATATGAAG	GAGAAGGGGA	AGGTGACAAG	AACATCTCCA	2880
GGAAGCCAGG	CAATGTGCTT	AAGACCCTGG	ATCCCATCCT	GATCACCATC	ATAGCCATGA	2940
GTGCCCTGGG	AGTACTCCTG	GGTGCAGTCT	GTGGAGTTGT	GCTGTACTGT	GCCTGTTGGC	3000
ACAATGGGAT	GTCAGAAAGG	AACCTATCTG	CCCTGGAGAA	CTATAACTTT	GAACTTGTGG	3060
ATGGTGTAAA	GTTGAAAAAA	GATAAACTGA	ACCCACAGAG	TAATTACTCA	GAGGCGTGAA	3120
GGCACGGAGC	TGGAGGGAAC	AAGGGAGGAG	CACGGCAGGA	GAACAGGTGG	AGGCATGGGG	3180
ACTCTGTTAC	TCTGCTTTCA	CTGTAAGCTG	GGAAGGGCGG	GGACTCTGTT	ACTCCGCTTT	3240
CACTGTAAGC	TCGGAAGGGC	ATCCACGATG	CCATGCCAGG	CTTTTCTCAG	GAGCTTCAAT	3300
GAGCGTCACC	TACAGACACA	AGCAGGTGAC	TGCGGTAACA	ACAGGAATCA	TGTACAAGCC	3360
TGCTTTCTTC	TCTTGGTTTC	ATTTGGGTAA	TCAGAAGCCA	TTTGAGACCA	AGTGTGACTG	3420
ACTTCATGGT	TCATCCTACT	AGCCCCCTTT	TTTCCTCTCT	TTCTCCTTAC	CCTGTGGTGG	3480
ATTCTTCTCG	GAAACTGCAA	AATCCAAGAT	GCTGGCACTA	GGCGTTATTC	AGTGGGCCCT	3540
TTTGATGGAC	ATGTGACCTG	TAGCCCAGTG	CCCAGAGCAT	ATTATCATAA	CCACATTTCA	3600
GGGGACGCCA	ACGTCCATCC	ACCTTTGCAT	CGCTACCTGC	AGCGAGCACA	GG	3652

Fig. 7

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NUMBER 032396-043

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NEUROPILIN ANTISENSE OLIGONUCLEOTIDE SEQUENCES AND METHODS OF USING SAME TO MODULATE CELL GROWTH

the specification of which (check only one item below):

☒ is attached hereto.

☐ was filed as United States application

Number _____

on _____

and was amended

on _____ (if applicable).

☐ was filed as PCT international application

Number _____

on _____

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
			__ Yes __ No
			__ Yes __ No
			__ Yes __ No
			__ Yes __ No
			__ Yes __ No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

60/082,791	April 23, 1998
(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NO. 032396-043

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.

PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)		ATTORNEY'S DOCKET NO. 032396-043
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